SPERMATOGENESIS IN PHILOSAMIA CYNTHIA.

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An investigation of spermatogenesis in *Philosamia cynthia*, a moth of the Saturnid family, was undertaken in the summer of 1905, at the suggestion of Professor Crampton, who for some years has been engaged in a statistical study of variation in this form.

Few researches have as yet been made in the spermatogenesis of Lepidoptera. Platner, in a paper published in 1886, appears to have been the first to describe the development of germ cells in this group of insects. Here, however, in his plates of *Pygæra* and *Sphinx*, he gave no details of chromosome numbers and divisions, but figured chiefly the development of cytoplasmic structures in the spermatid. Among other writers who have concerned themselves principally with the cytoplasmic aspect of development, may be mentioned Meves ('97) and La Valette ('97). Munson ('06) figures a few chromosome groups in connection with an extensive account of the development of achromatic structures in the spermatogenesis of *Papilio*. Toyama's paper on the silkworm I have not been able to obtain. The observations of Miss Stevens, published in the past year, upon the spermatocytes of the butterflies *Cacœcia* and *Euvanessa*, will be referred to later.

MATERIAL.

The life history of *Philosamia* is, briefly as follows: The eggs are laid the early part of June; develop into larvæ which pupate in September, and remain in the pupal stage until their emergence as moths the following June. The development of the spermatocytes takes place in the pupa. The testes are kidney-shaped bodies, about one eighth inch long, lying within the body cavity directly beneath the abdominal tergum. They are enveloped by voluminous yellow fat bodies, from which they can be readily distinguished by their lighter color, and compact shape. The testis (Fig. 1) is divided into four lobes, by three thin sheets of

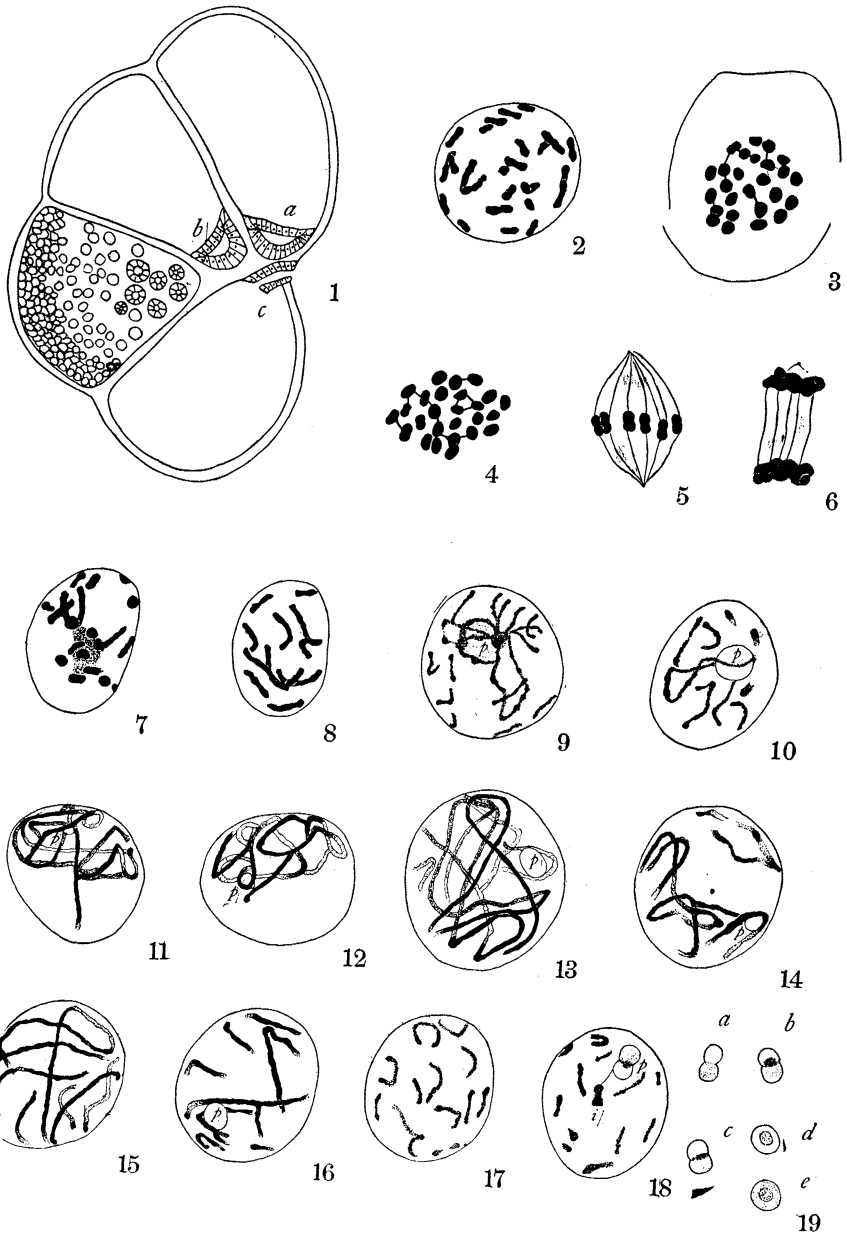


FIG. 1. Diagram of horizontal section through testis (enlarged 20 diameters) showing arrangement of cysts in the lobes. Openings of vas deferens cut obliquely at *a* and *b*, longitudinally at *c*.

FIG. 2. Early prophase of last spermatogonial division.

FIGS. 3, 4. Polar view of last spermatogonial metaphase.

FIGS. 5, 6. Side views of metaphase and of telophase (optical sections).

FIGS. 7–10. Various stages in spireme formation; appearance of plasmosome.

FIGS. 11, 12. Concentrated stage of spireme.

FIG. 13. Height of spireme stage.

FIGS. 14–16. Stages in breaking up of spireme.

FIG. 19, *a–e*. Various views of plasmosome from nuclei similar to Fig. 18.

connective tissue, which extend from the outer or convex side of the organ, and converge towards the "hilus," where each lobe opens into the sperm duct. The mature cells lie near this point. Spermatogonia are massed closely together at the opposite end, and the cells in the growth stage are grouped together in rounded cysts which lie free in the lumen of the lobe.

Methods.

Upon removal of the dorsal abdominal wall, the testes were quickly dissected out and transferred immediately to the fixing fluid. Corrosive-acetic, Gilson's alcohol-chloroform-acetic, and Flemming's fluids were used. The two former fixing agents proved especially good for spireme stages, but achromatic structures were more clearly defined with Flemming's fluid. The sections were stained with iron hæmatoxylin, with which it was possible to differentiate the plasmosome, or true nucleolus, from the chromatic nucleolus. Thionin was also used, but did not differentiate so clearly.

The figures for this paper are, with the exception of Fig. 1, from camera drawings made with compensating ocular No. 8, with a tube length of 160 mm., and $\frac{1}{12}$ oil immersion lens. They were enlarged $2\frac{1}{2}$ diameters with a drawing camera, corrected from the original, and then reduced one half in the final plates.

General Development.

Owing to the long period of development, it is not possible to find all stages of germ cells in any one testis. In material fixed during the winter, the series ranges from spermatogonia to perhaps only the first spermatocyte prophase, while in testes fixed in early June, about one week before emergence, nearly all of the cells are transformed into spermatids and spermatozoa.

There are several interesting points of general development to be observed in the winter material. A varying amount of disintegration takes place in the cells. A few first divisions appear in the autumn pupæ, but at a later period none are found, so that it is probable that these are precocious first divisions, which are followed by disintegration, while the permanent division stages appear in the spring. This observation differs from that of Wilcox, who found in *Caloptenus* that "if cells reach the spermatocyte stage they complete their course."

The cells in the growth stage also show a certain amount of disintegration, when the chromatin appears to be concentrated into one or more spherical bodies resembling yolk granules. Another point observed is that the spermatogonia along the periphery proliferate inwards a new growth at one point, forming a compact mass, the later development of which may go to make up the deficiency caused by disintegration.

In several preparations of very late stages, made in the early summer, where nearly all the cells were in the spermatid phase, small groups of giant spermatogonia were observed near the periphery of the testes. The stages ranged only from prophases to anaphases. Metaphase groups were most frequent, and several clear counts were obtained giving twenty-six chromosomes, the normal spermatogonial number. Montgomery found a similar condition in the spermatogenesis of *Peripatus*. All these giant spermatogonia were in mitosis, *i. e.*, from late prophase to anaphase. No earlier nor later stages were observed. Montgomery found that these cells were more numerous in cysts showing disintegration, and concluded that they were "hypertrophied spermatogonia, whose mitosis proceeds normally as far as the anaphase, when atrophy begins." In my preparations I have not observed that the presence of giant spermatogonia is correlated with disintegration in the cysts.

Spermatogonia.

The chromosomes of the last spermatogonial metaphase can be quite clearly seen in polar view, connected in many cases by what appear to be thin black threads (Figs. 3, 4). They are rounded bead-like bodies of approximately the same size. When turned obliquely they show a bipartite form, preparatory to the last spermatogonial division, and consequently appear larger. In the best polar views of the metaphase, twenty-six chromosomes can be seen. Only in cases where the chromatin elements are more concentrated, and hence difficult to count, does the number appear less. A side view of the equatorial plate, an optical section (Fig. 5), shows six bipartite chromosomes, arranged in a regular line at the center of the spindle. Many side views with a much larger number were seen, but this is typical of the regu-

larity of form and division of all the chromosomes. The nuclear membrane disappears at the metaphase. In telophase, the chromosomes are crowded into a dense mass, in which it is difficult to determine the outlines of individual ones (Fig. 6).

After the daughter cells have formed, and the nuclear membrane again encloses the chromosomes, the nucleus increases in

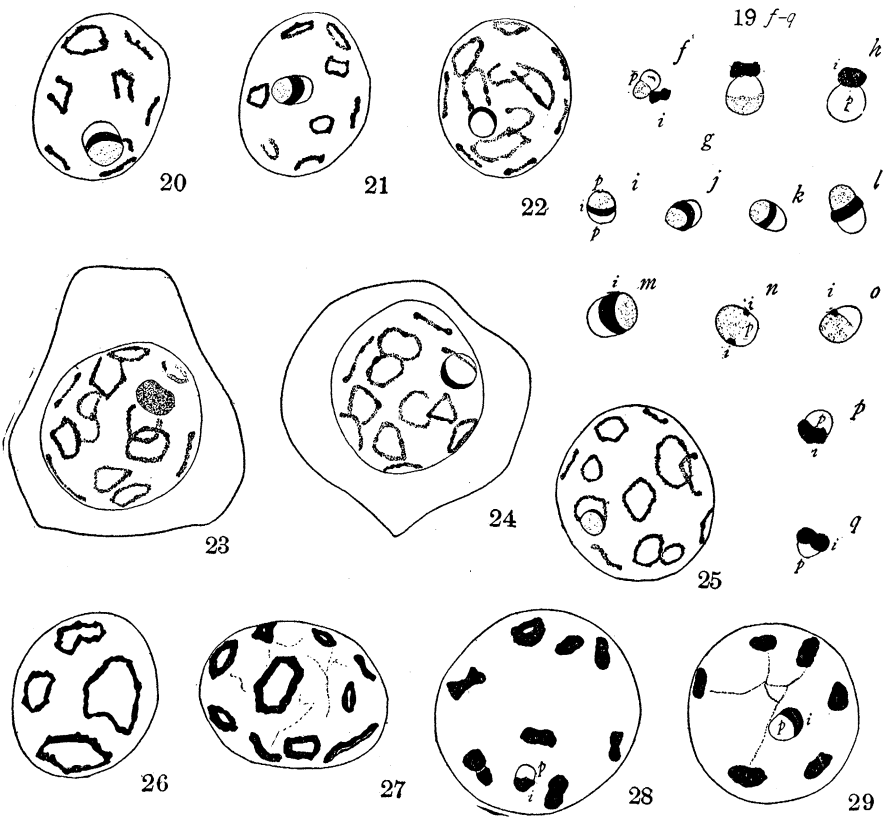


FIG. 19, *f–q*. Various views of nucleoli during growth period, showing relation of idiochromosome to plasmosome.

FIGS. 20, 21. Early ring stage—idiochromosome attached to plasmosome.

FIGS. 22–25. Four views of later stage—showing twelve rings and nucleolus.

FIGS. 26–29. Contraction of rings into chromosomes.

size, and the chromosomes spread out into the cavity. Figs. 7, 8 show a transition from the characteristic round or oval chromosomes of the spermatogonia into rods of varying length and

uneven contour. Several of the chromosomes are grouped around a dark gray mass near the center of the nucleus, in which I believe, from what occurs later, the plasmosome appears.

The chromosomes seem to transform from rods, into longer and thinner skein-like pieces, with rougher outline, and lighter staining capacity. In Fig. 9 some of these pieces are seen to form a spireme, and this, like the chromosomes in the preceding figure, is centered around a large gray-staining mass, which is seen in Fig. 10 to be a definite plasmosome.

Spireme Stage.

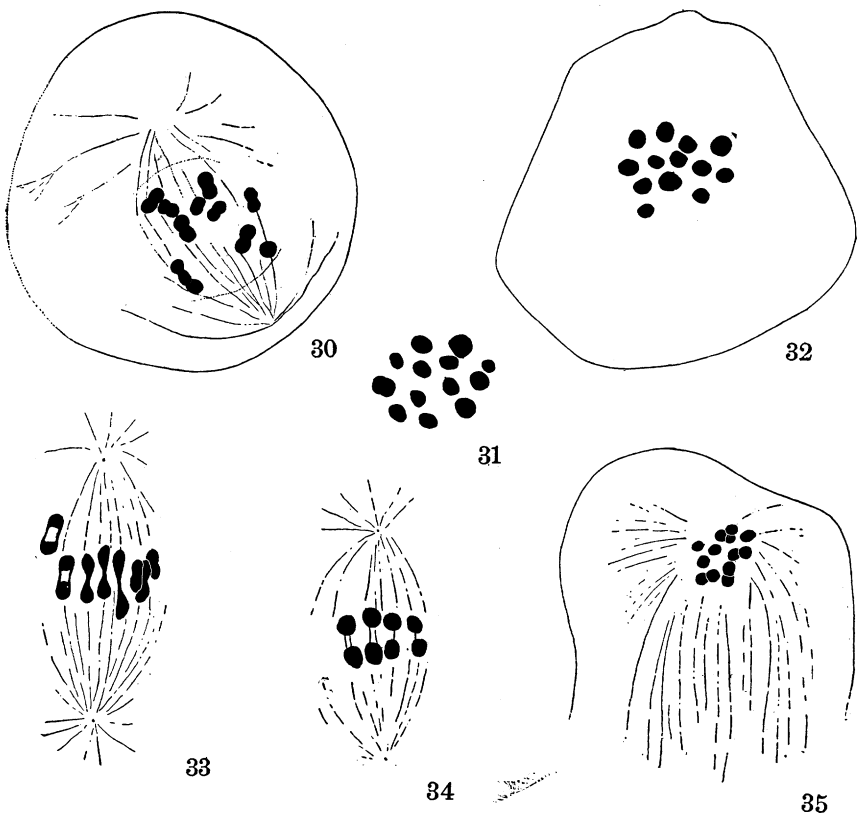
Figures 11, 12 are characteristic of the next definite stage. The chromatin pieces now form a close intricately-coiled spireme, the mass being contracted against one side of the nucleus, nearest the greatest amount of cytoplasm, as Montgomery described in *Syrbula*. The spireme is not continuous, for several free ends are seen in sections which give a complete focus of the nuclei; but it is impossible to determine the number of threads which form it. A plasmosome is seen, entangled in the meshes of the spireme. The threads are thicker and smoother in outline than in Fig. 9, and stain black even in sections which in other respects are light in color.

The question of synapsis was carefully studied in stages from 7 to 11, but I have not been able to obtain decisive evidence regarding the nature of the process. I was unable to find evidence that the chromatin rods unite definitely two by two in forming the spireme, but that they do unite in some manner seems clear. Nowhere have I found stages which might be interpreted as parasynapsis, or side by side union of the chromosomes, such as has been figured by the Schreiners, and other observers, nor could I discover that in the spireme the threads show a longitudinal split.

After contraction at one side of the nucleus, as shown in Figs. 11, 12, the spireme spreads out to occupy the entire nuclear cavity, which increases in size. Fig. 13 represents an unusually large nucleus, where the coiling of the threads, and the plasmosome, are distinctly seen.

We may speak of this condition as the height of the spireme stage. From this point onward, the spireme threads appear to

break up into shorter elements, and to become somewhat more disentangled from one another, as figured in Nos. 14 and 15. In the latter the plasmosome is not in the plane of section. In Fig. 16 this fragmentation has become more marked, until in Fig. 17, the spireme is transformed into thirteen pieces, of irregular



FIGS. 30–35. First spermatocyte division.
FIG. 30. Prophase (two chromosomes lacking in this section).
FIGS. 31, 32. Polar views of metaphase groups showing 13 chromosomes.
FIGS. 33, 34. Side views of anaphase (not all the chromosomes are shown).
FIG. 35. Telophase.

shape and outline, which are more granular and stain less intensely, than the previous stages. (The plasmosome does not appear in this section.) In Fig. 18 the limit of spireme fragmentation has been reached. Thirteen chromatin elements appear, one of which is denser than the others. The plasmosome is characteristically bipartite in this and later stages.

There is a very constant difference in the appearance of the two parts of the plasmosome. One half is clear and transparent, the other, slightly granular, and stains a deeper gray. Various views of it are shown in Fig. 19, *a* to *e*, drawn from nuclei of the same stage as Fig. 18. Three side views are given in Fig. 19, *a–c*; in the two latter the gray half seems more darkly granular at its region of union with the other. In end view, *d*, *e*, only one part is seen in outline, so that the structure appears as a single sphere, but a smaller granular area is found in the center, which seems to be nothing but the granular region indicated in *b*, seen through the clear half of the plasmosome.

The intervening history from the breaking up of the spireme into thirteen elements, and the appearance of a double plasmosome, up through the stage when rings are formed in the growth period, will be passed over for the present, to a consideration of the

Maturation Divisions.

In prophase of the first maturation division, the chromosomes appear regularly bipartite, and approximately equal in size (Fig. 30), placed irregularly upon the spindle, in preparation for metaphase. (The section lacks two of the typical number.) Many very clear metaphase groups, seen in polar view, show invariably thirteen chromosomes. Two incomplete sections (Figs. 33, 34) give views of typical anaphases, where division always appears equal.

Polar views of the second metaphase (Figs. 36, 37) have the same grouping and appearance as in the first division, the only difference being in the size of the chromosomes. In a late anaphase of second division (Fig. 38), the chromosomes of each group are approximately similar in size, and in none of the anaphases studied have I seen a case of unequal division. In early telophase the chromosomes are crowded together, and difficult to count, but several counts from polar view, or slightly oblique, showed the usual number, thirteen. Figures 39–41 are various views, all showing thirteen chromosomes as a result of second division. Fig. 42 shows the characteristic lengthening of the spindle in this division.

From the foregoing facts it is clear that in *Philosamia* there is

no odd or "accessory" chromosome, and since there seems to be also no unequal division of chromatin material, there is no element that can be distinguished as an idiochromosome-pair in the metaphase. There is, however, reason to believe that one of the bivalents differs from the others during the growth period in such a way as to indicate that it is to be identified as an equal pair of idiochromosomes, comparable with that described by Wilson in the case of *Nezara*.

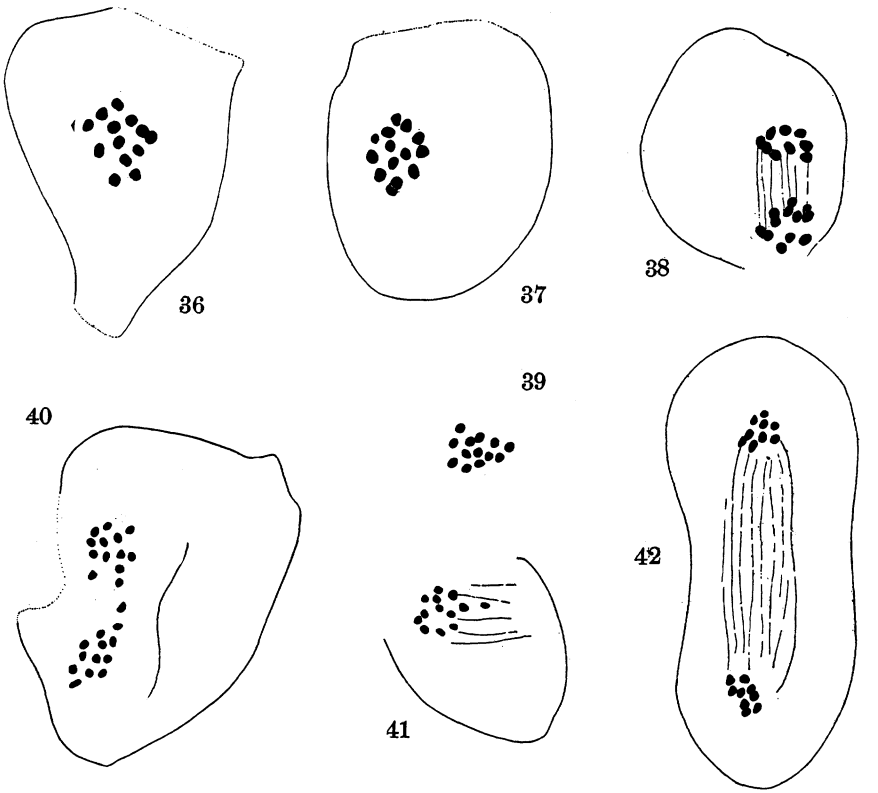
Ring Stage.

Having found twenty-six chromosomes in the spermatogonia. thirteen broken chromatin elements after the spireme stage (Figs. 17, 18), and thirteen chromosomes in maturation metaphases, I naturally expected to find that in the ring stage resulting after breaking of the spireme, thirteen rings were present. I found, however, only twelve, in a very large number of cells which I examined (Figs. 22–25). No clear case showing thirteen was observed.

My next step was to study the nucleolus present in the ring stage, to see if it might be different from the earlier double plasmosome, in containing some chromatin material. I found the double plasmosome, previously noted in Figs. 18 and 19, *a–e*, and in addition a deep black crescent-shaped band attached around the middle, its concave edge directed always toward the darker portion of the plasmosome as in Figs. 20 and 21, and 19, *m*, *n*, *o*, the two latter being views of the reverse side of the nucleolus, showing that the chromatin does not completely encircle the plasmosome. This chromatin band I interpret as equivalent to the two equal idiochromosomes found by Wilson in *Nezara*, the band thus being bivalent, as is each of the rings, and the number of chromatin elements thus forming the correct reduced number thirteen.

How, and when, does this chromatin band become associated with the plasmosome? The evidence seems to show that it is derived from one of the chromatic elements — in the case of Fig. 18, probably the darker, broader mass — and attaches itself at this stage to the plasmosome. In Fig. 19, *f*, is represented a plasmosome with the chromatin mass drawn up near it, preparatory

to attachment. The other chromatin elements in this nucleus are all irregular and feathery in outline, as in Fig. 18. Fig 19, *g*, *h*, is an end view of the same stage, showing a larger structure, with the same characteristic chromatin mass. In *i* and *j* this has become attached to the plasmosome. From the broken chromatin rods the rings are formed, apparently by a bending around of the rods; but the exact manner of the change seems very dif-



FIGS. 36–42. Second SpermatocyteDivision.
FIGS. 36, 37. Polar views of metaphase groups with 13 chromosomes.
FIG. 38. Anaphase.
FIG. 39. Polar view of telophase.
FIGS. 40, 41. Late anaphase groups, side view, showing 13 chromosomes.
FIG. 42. Telophase (not all the chromosomes appear).

ficult to determine (Figs. 20, 21). In this stage, which lasts throughout the winter, the double nucleolus with its chromatin band, is a constant factor. The rings are irregular in size and

form, very granular in appearance, and stain less deeply than the spireme.

Concentration of Rings.

This is the next well-marked stage. The threads forming the rings become thicker, rougher in outline, and more deeply staining (Fig. 26), and as they thicken their circumference decreases until the originally large central space is reduced to a minute cavity, which is finally closed up altogether, and a chromosome results. Fig. 27 shows various stages in concentration. Chromosomes as shown in Fig. 28 and 29 succeed this until the at first irregular elliptical masses have assumed the smooth dumb-bell-like form seen in the first prophase stage (Fig. 30).

During concentration of the rings, the idiochromosome also appears to thicken, becoming shorter and broader, so that it has a smaller surface of contact with the plasmosome (Fig. 19, *p*, and 28). In Fig. 19, *q*, from an early prophase, it has the smooth bipartite form common to the other chromosomes. From this time, the plasmosome disappears, and the idiochromosome is indistinguishable from the others.

Conclusion.

The presence of a double idiochromosome in *Philosamia* connects it in this respect, with *Euvanessa* and *Cacœcia*, two species of butterflies studied by Miss Stevens ('06), who finds an equal pair of idiochromosomes, or "*sometimes a two lobed body*," . . . "whose only apparent peculiarity is its condensed form during growth."

In the case of the moth the idiochromosome appears single from the time of its first appearance, but it would seem that it is a *bivalent* body, in just the same way that the rings and resulting chromosomes are bivalent. This bivalence has its origin in all probability, in the prespireme stage.

Philosamia thus lies at the opposite end of a series, from *Nezara*, where the equal idiochromosomes do not unite until after the first division. An intermediate stage is represented in *Brochymena* (Wilson, '05), where the idiochromosomes, in this case unequal, lie at first separated, but later united, in the growth period. Wilson concludes for this form that, "when only one

chromatin nucleolus is present, it is to be considered as a bivalent body, arising by fusion or synapsis of the two idiochromosomes." In *Brochymena*, however, they separate again before the first division.

I wish to acknowledge my indebtedness to Professor Crampton for suggestions and material and also to Professor Wilson for kindly supervision and corrections, and reading of manuscript.

Summary.

1. The spermatogonia contain twenty-six chromosomes, of approximately the same size and shape.

2. There is a definite spireme stage with a simple plasmosome.

3. The spireme segments into thirteen parts, of which twelve form rings, the thirteenth becoming attached as a chromatin mass to the plasmosome, which at this stage is double.

4. In the growth period, when the twelve rings are definitely formed, the chromatin mass is bent in a crescentic band around the plasmosome, forming a chromosome nucleolus.

5. This band represents a pair of idiochromosomes, and is bivalent like the rings, but always appears as a single body.

6. First and second metaphases show thirteen chromosomes. Divisions are equal, so that the spermatids contain similar chromosome groups.

Columbia University,
March, 1907.

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